

LabLink

Laboratory Information from the Michigan Department of Community Health Bureau of Laboratories

Vol. 6 No. 1 Summer 2000

The Select Agent Rule

Frances Pouch Downes, Dr.P.H. Laboratory Director

Increasing national concern regarding the use of microorganisms, toxins and chemicals as agents of bioterrorism resulted in the Antiterrorism and Effective Death Penalty Act of 1996. Section 51.1 of the legislation resulted in the final rule, "Additional requirements for facilities transferring or receiving select agents" (42 CFR Part 72.6, Federal Register Oct. 24, 1996), commonly referred to as the Select Agent Rule. The Select Agent Rule is designed to ensure that infectious agents and toxins are shipped to institutions or individuals equipped to handle them appropriately and legitimate reasons to use them.

The Select Agent Rule creates a system of safeguards which provide information on the location of certain potentially hazardous agents as they are transferred, tracking the acquisition and transfer of these agents and establishing a process for alerting authorities if an unauthorized attempt is made to acquire these agents. The components of the rule are as follows: a comprehensive list of select agents (see insert page 2), a mechanism for registering facilities, transfer requirements, verification procedures, agent disposal and research and clinical exemptions.

Clinical laboratories utilizing these agents for diagnostic, reference, verification or proficiency testing are exempt from the provisions requiring tracking, facility registration, etc. Use of the select agents in the clinical laboratory must be directly related to the health of the human or animal. In addition to traditional diagnostic testing settings, testing involving public food safety, animal health, evidence for law enforcement and detection of bioterrorism agents are also exempt. Clinical laboratories designated as A level laboratories in the

Michigan Laboratory Response Network and public health laboratories at the B and C levels can, therefore, transfer specimens and suspect isolates of bioterrorism agents. If the CLIA certified laboratory transfers specimens to a non-exempt facility (e.g., research or manufacturing facility for non-patient related testing) the CLIA laboratory must complete form EA 101 using their CLIA certification number in lieu of a Select Agent Registration number.

All transfers of select agents must be in accordance with federal regulations for shipping infectious substances (a.k.a., dangerous goods, etiological agents). See specific instructions for shipping in the following article.

Please note that shippers are responsible for the appropriate packaging material and completion of the "Shippers Declaration for Dangerous Goods." MDCH supplies shipping containers which comply with the UN 6.2 standards for shipment of bacterial and viral isolates. Laboratories submitting isolates of bacteria or viruses utilize these materials according to the instructions provided and complete the shipping declaration. Your cooperation is appreciated. If you have questions concerning shipping or the select agent rule contact Sam Davis at (517)335-8074. Additional Resources:

Richmond, J.Y. and R.W. McKinney (eds.) 1999. Biosafety in Microbiology and Biomedical Laboratories. CDC/NIH. (www.cdc.gov/od/ohs/biosfety/bmbl4/bmbl4toc.htm)

Instructions for Completion of Shippers Declaration for Dangerous Goods

Sam Davis, Office of Quality Assurance

Infectious Substances Require a "SHIPPER'S DECLARATION FOR DANGEROUS GOODS"

Infectious substance means a viable microorganism, or its toxin, that causes or may cause disease in human beings or animals and includes those agents listed in 42 CFR 72.3 and any other agent that causes or may cause severe, disabling or fatal disease. The terms "infectious substance" and "etiologic agent" are synonymous. Each package must have a properly completed shipper's declaration for dangerous goods affixed to the outside of the mailpiece with the following information and statements completed by the shipper; (see sample form on page 3)

- 1. **Shipper**: Your Name, Address & Phone Number
- 2. **Consignee**: Our Name, Address & Phone Number
- 3. **Transport Details**: Airport of departure & airport of destination. Delete "Cargo Aircraft Only"
- 4. **Shipment Type**: Delete "Radioactive"
- 5. **Proper Shipping Name:** "Infectious Substance, Affecting Humans (Specify Organism Name In Brackets)" (Note: This information must also be on the label above the Infectious Agent sticker on the outside carton.)
- 6. **Class**: 6.2
- 7. **UN or ID number**: UN2814 (Note: Packing Group & Subsidiary Risk: **Leave Blank** These do not apply to infectious substances.)
- 8. Quantity & Type of Packing: "One fiberboard box __ X ____mls" (Note: This information [total volume] must also be on the label above the Infectious Agent Label on the outside carton.)
- 9. **Packing Inst.:** 602 (Note: Authorization: **Leave Blank**)
- 10. Additional handling information: "This package prepared according to the provisions of IATA/IACO. Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made." (Note: This means you have advised the consignee that they will be receiving the items listed on the declaration per 42 CFR 72.3)
- 11. **24 hour emergency contact number:** Your Agencies Number
- 12. **Name/Title of Signatory:** Your name/title

Place and Date: Your city & date of shipment Signature: Your signature

The shipper is responsible for being sure that their package is in compliance with current regulations. Keep one copy of this form for your records for thirty days

When attaching the plastic envelope to the outside of the package, do not seal it completely. Leave the narrow protective strip on the envelope so that form may be reviewed/replaced by the company transporting your specimen.

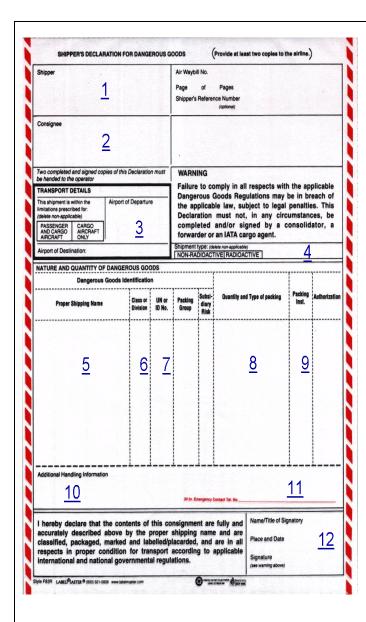
These forms are currently being provided in Units 3, 9, 13, and 42 provided by MDCH. If you have older Units - forms and envelopes for attaching to the outside of the mailpiece may be requested by phone (517-335-9867), fax (517-335-9039, or E-mail: DietzR@state.mi.us. If further information is desired contact the U.S. Postal Service @ 202 - 268 - 6249, IATA @ 800 716-6326, FedEx @ 800-463-3339, Ext. 81, or: http://www.cdc.gov/od/ohs/biosfty/shipregs.htm

Select Agents

Bacterial Agents Rickettsial Agents Coxiella burnetii Bacillus anthracis Brucella spp. Rickettsia spp. Clostridium botulinum Francisella tularensis Toxins Pseudomonas mallei Abrin Pseudomonas pseudomallei Aflatoxins Botulinum toxins Yersinia pestis C. perfringens **Fungal Agents** (epsilon toxin) Coccidioides immitis Conotoxins Diacetoxyscirpenol Viral Agents Ricin Arboviruses Saxitoxin Ebola virus Shigatoxin

Lassa virus Marburg virus

Tick-borne encephalitis virus complex Variola major and minor viruses



New Employees and Retirements

The Bureau of Laboratories would like to welcome seven new employees. Jayshree Patel, Kelly Scott and Kathleen Russell have joined the Microbiology section as laboratory technicians in the Mycobacteria/Mycology unit. Teresa Miller has joined the Molecular Biology section as a laboratory technician. Dean Walker joins the Health Risk Assessment section as a laboratory scientist. The Newborn Screening section has hired Penny Crawford as a data coder and Leah Theisen as a laboratory technician.

The bureau bids farewell to two employees. Robert Lee of the Media unit and Dr. Steve Hsu of the Virology/Immunology section have retired from state service. The bureau wishes them the best in their new endeavors.

Virology Section Gets New Director

Duane Newton, Ph.D., has been appointed the new manager of the Virology/Immunology section for the Bureau of Laboratories at MDCH. Newton received his doctorate in Microbiology and Immunology from the University of Dayton, Ohio, in 1993. Newton has held a variety of post-doctoral positions including fellowships as a research microbiologist, National Institute of Health training fellow and American Heart Association fellow. Since 1998 Newton had been in an American Society for Microbiology sponsored clinical and public health microbiology training program at the University of Rochester (New York) Medical Center. Newton welcomes questions and comments. He may be reached at (517) 335-8099.

CDC Announces New Area PulseNet Laboratories

MDCH, bureau of laboratories, molecular biology section has been selected as one of the new PulseNet area laboratories according to Bala Swaminathan, Ph.D., Chief of the Foodborne and Diarrheal Diseases Laboratory Sections at the Centers for Disease Control and Prevention. The Michigan laboratory was one of three chosen from 16 applications submitted to the Association of Public Health Laboratories for consideration.

PulseNet is a national network of public health laboratories, CDC, USDA and the FDA. It was established by CDC to perform standardized DNA fingerprinting on isolates of foodborne bacterial pathogens. The current method used for fingerprinting is pulse field gel electrophoresis (PFGE). The purpose of PulseNet is to rapidly detect outbreaks through surveillance of selected pathogens and to assist in investigations of outbreaks caused by other foodborne bacteria by allowing comparison of isolates from multiple states. MDCH has participated in the PulseNet system since 1998.

As an area laboratory, MDCH will coordinate multi state outbreak investigations, provide PFGE services to neighboring states that do not have the capacity, provide training for other PulseNet laboratories and participate in the development and evaluation of new fingerprinting methods.

PulseNet area laboratories are required to routinely perform PFGE on isolates of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. MDCH routinely performs PFGE on the required organisms as well as all isolates

of Salmonella sp. sero. Typhimurium and Shigella sonnei.

Quirky Bugs ...

Biological Warfare: The Plague Threat

Sandip Shah, MS, MT(ASCP), Reference Bacteriology

At 4:00 AM on a warm and rainy September (1994) morning in Lansing, Michigan the phone rang. My mother-in-law was on the other end calling from Surat, in western India, to tell us that she was fleeing the town along with fifty thousand other residents. A epidemic of pneumonic plague was detected in the town. Thousands of people were sick and hundreds were in their deathbeds. All major routes were blocked and looked like parking lots. No trains, buses or planes were coming to the town from outside due to the scare and panic. Military personnel were patrolling the streets in their suits, masks distributing medicines to the remaining citizens.

Yersinia pestis is the causative agent of plague. Given the availability of *Y. pestis* around the world, capacity for its mass production and aerosol dissemination as a biological weapon is of great concern.

Although the example above resulted because of endemic disease, experts say it is only a matter of time before terrorists strike with such a biological weapon. The Medical and Public Health Management Working Group on Civilian Biodefense has developed consensus based recommendations for measures to be taken by medical and public health professionals following the use of plague as a biological weapon against civilians. The group has concluded that an aerosolized plague weapon could cause fever, cough, chest pain and hemoptysis with signs consistent with severe pneumonia one to six days after exposure. Rapid evolution of disease would occur in the two to four days after symptom onset. This would lead to septic shock with high mortality. Without early treatment, this could cause disease and death in sufficient numbers to cripple a city or region. Early treatment and prophylaxis with streptomycin, gentamicin or the tetracycline or fluoroguinolone classes of antimicrobials would be advised. The US licensed, formaldehyde killed whole bacilli vaccine was discontinued by its manufacturers in 1999 and is no longer available.

The role of the Microbiology laboratory-

The microbiology laboratory can play a critical role. In the event of an announced attack, the microbiology laboratory can aid in the rapid diagnosis of possible cases of disease. By continuous awareness of the potential for plague in any clinical sample received, the microbiology laboratory also can detect unannounced attacks which may present as illness or death of unknown origin.

Y. pestis is a member of the Enterobacteriacae. It is a

nonmotile, gram negative bacillus or coccobacillus. It will demonstrate bipolar staining with Wright, Giemsa or Wayson stains. Y. pestis is a lactose nonfermenter, and is urease and indole negative. It grows optimally at 28°C on blood agar or MacConkey agar, typically requiring 48 hours for observable growth. Colonies are initially much smaller than other Enterobacteriaceae and may be overlooked. The first clinical or laboratory suspicion of plague must lead to immediate notification of the hospital epidemiologist or infection control practitioner, health department and the local or state health laboratory

In the unlikely event that a cervical bubo is present in pneumonic plague, an aspirate obtained with a 20-gauge needle and a 10-mL syringe containing 1-2 mL of sterile saline for infusing the node may be cultured. Cultures of sputum, blood or a lymph node aspirate should demonstrate growth approximately 24 to 48 hours after inoculation. Most microbiology laboratories use automated or semiautomated bacterial identification systems. Some systems may misidentify *Y. pestis*. In laboratories without automated bacterial identification, as many as six days may be required for identification.

Laboratory Levels and Procedures-

Handling of samples and decontamination All samples containing *Y. pestis* should be handled in a biological safety cabinet. Specimen handling should be performed using universal precautions by an experienced microbiologist. Commercially available household bleach solutions contain 5.25 percent hypochlorite and when diluted 1:10. are effective in routine decontamination of surfaces and instruments after working with *Y. pestis* All work surfaces must be wiped after use with this solution. Items such as pipettes, slides, loops and needles should be immersed overnight in decontamination solution and then autoclaved.

The Level A laboratories are designed to presumptively identify *Y. pestis* isolates from clinical specimens. These procedures should be performed in microbiology laboratories that utilize Biosafety Level 2 practices. The purpose is to obtain appropriate specimens for culture and rapid detection of the organism from clinical specimens. Sample collection from plague patients is determined by the form of the disease and its progression. Any positive smear or culture result should be promptly reported to the patient's physician and to your state or local health department. Positive smears and isolates should be immediately forwarded to the nearest Level B laboratory for confirmation. In most areas this will be the state public

health laboratory.

The Level B & C laboratory procedures will provide rapid confirmation of suspect organism. Laboratories that perform these procedures should also be proficient in Level A procedures. Confirmatory tests of *Y. pestis* include direct fluorescent antibody staining for *Y. pestis* F1 antigen, specific bacteriophage lysis test, antimicrobial susceptibility profile by E-test, biochemical tests, mouse inoculation for recovery, separation of cellular proteins, immunoblot, plasmid profiling and pulsed field gel electrophoresis.

Level D laboratories (CDC) serve as national and international resources for the development and evaluation of new technologies for the laboratory diagnosis and epidemiologic investigation of plague. These laboratories create and maintain collections of reference isolates, clinical and environmental isolates and patient and environmental specimens for research and development. Other responsibilities of Level D laboratories include serology tests, immunohistochemical staining, environmental specimen evaluation, reagent preparation and standardization and proficiency program management.

References:

- 1. CDC/APHL. Laboratory protocols for Bioterrorism Response Laboratories, 1999.
- 2. CDC/FDA. Biological Warfare and Terrorism, The Military and Public Health Response, 1999.
- 3. World Health Organization. International Health Regulations. 3rd ed. Geneva, Switzerland: WHO, 1983:26-9.
- 4. Desai D, Samuel I. Surat flounders against medical crisis. The Indian Express 24 September 1994.
- 5. Centers for Disease Control and Prevention. Human plague—India, 1994. MMWR 1994;43:689-91.

For More information on Internet - visit http://www.cdc.gov

West Nile Virus – Can We Detect It?

Duane W. Newton, Ph.D. Director, Virology/Immunology Section

As a follow-up to the article on arbovirus testing, MDCH would like to provide some information regarding national surveillance and detection efforts for West Nile Virus (WNV). In February 2000, CDC made funds available to certain state, local and municipal health departments located along the Atlantic and Gulf coasts, to enhance WNV surveillance and laboratory detection capabilities. In June 2000, similar funds were made available, through a competitive grant to 29 other health departments in the U.S. because of concerns about the spread of the virus. A cooperative proposal submitted by the MDCH Bureaus of Laboratories and Epidemiology, in collaboration with the

Michigan Department of Agriculture and Michigan State University Department of Entomology, has sought to develop mechanisms for active human and passive animal (primarily dead birds) surveillance, as well as enhance our capacity for specific detection of WNV in the laboratory. There are currently no WNV specific tests available in the state, so specimens from suspected cases would be sent to CDC or another appropriate reference laboratory. Since WNV and SLE are from the same family of viruses, they share enough antigenic similarity that reagents used in the antibody capture assay for SLE will cross react to detect WNV specific antibodies. Positive results obtained from antibody capture assays, at MDCH, are sent to CDC for specific confirmation. Future updates regarding new developments in WNV and arbovirus testing at MDCH will be released in future issues of the LabLink.

Mycobacterium bovis in Michigan

Barbara Robinson-Dunn, Ph.D. Director/Microbiology Section

In 1994, a free-ranging deer from Alpena County infected with Mycobacterium bovis was identified. Since that time, MDCH has provided diagnostic microbiology laboratory testing services to determine the frequency of *M. bovis* infection in deer harvested from northeast Michigan. This work is coordinated with the Departments of Natural Resources (MDNR) and Agriculture (MDA) and the College of Veterinary Medicine at Michigan State University (MSU) to reduce the incidence of bovine tuberculosis in Michigan. MDNR transports suspect harvested deer to the animal health diagnostic laboratory at MSU. Following necropsy and preliminary histologic examination of the tissues, suspect positive tissue samples are sent to MDCH for microscopy and culture to determine the presence of *M. bovis*.

Since 1994, the MDCH mycobacteriology laboratory has tested a total of 521. A total of 329 other animal specimens have been tested as part of surveillance activities and research projects to determine whether *M. bovis* occurs in other species and whether those species can serve as vectors in the dispersal of this agent.

MDCH has provided extensive laboratory testing to determine whether there has been spread of *M. bovis* from wildlife to humans. Since 1997, the MDCH Mycobacteriology laboratory has identified *M. bovis* in specimens from six different human patients. DNA fingerprinting of the deer and human isolates has shown that the isolates are not similar. Thus, laboratory studies have been unable to demonstrate the spread of *M. bovis* from deer to humans.

MDCH will continue to work closely with MDNR, MDA and MSU to safeguard Michigan's residents from the health threat of bovine tuberculosis. The department will continue to provide testing services and surveillance to monitor bovine, as well as, human tuberculosis, in a cooperative effort to eliminate tuberculosis from Michigan.

after the onset of symptoms.

Individuals responsible for disease reporting should have a copy of "Laboratory–Disease Reporting" which lists all reportable conditions and the time frame in which they must be reported. For copies of this reference, please contact the Communicable Disease and Immunization Division at 517-335-8165.

3-month Old Dies After Michigan Lab Fails to Report Positive HBsAg

Nancy Fasano Communicable Disease and Immunization Division

In December, a three-month-old Michigan infant died from acute hepatitis B. MDCH staff reviewed provider and hospital records. Staff in the practice where the infant's mother received her prenatal care, as well as staff at the hospital where the infant was born, were interviewed. The MDCH review revealed that the infant's mother was positive for hepatitis B surface antigen (HBsAg) during her pregnancy. Neither the laboratory that conducted the test nor the prenatal care provider reported the test results to the local health department. The mother was chronically infected with hepatitis B and tested as required by Michigan's communicable disease rules. In addition, the test results were communicated inaccurately to the hospital where the baby was born.

The hospital had stopped giving all newborns the first dose of hepatitis B vaccine before discharge because of a concern raised about the preservative thimerosal in hepatitis B vaccine (thimerosal-free hepatitis B vaccine is now widely available). Because the information from the prenatal care provider incorrectly indicated that the infant's mother was negative for hepatitis, the infant received no hepatitis B vaccine and no hepatitis B immune globulin (HBIG).

If the laboratory or the prenatal care provider had reported the test results as required, the local health department could have followed up with the hospital prior to delivery to assure that hospital staff were aware of the woman's positive HBsAg status. The infant would have received appropriate prophylaxis. The infant became ill at about three months of age and died less than two weeks

Arbovirus Testing at MDCH

Duane W. Newton, Ph.D. Director, Virology/Immunology Section

During the warmer months of summer there is a heightened awareness of infections caused by arboviruses that can be transmitted through mosquito bites. Arboviruses are a group of animal viruses that are maintained in nature through transmission between susceptible hosts by blood feeding arthropods (e.g.,mosquitoes, ticks, etc.). This occurs through complex life cycles that involve a nonhuman primary vertebrate host and a primary arthropod vector. Humans and domestic animals can become infected but are usually dead-end hosts, not contributing to the transmission cycle.

The majority of arboviral infections in humans are asymptomatic or may result in a nonspecific flu-like syndrome. Symptom onset may be subtle or sudden, with fever, headache, myalgias, malaise and occasionally extreme exhaustion. In a small portion of infected patients there is a progression to encephalitis, with a fatal outcome or permanent neurological complications resulting from the infection.

Arboviruses are distributed worldwide, but three major viral agents are most likely to cause disease in Michigan: Eastern Equine Encephalitis virus (EEE), California Encephalitis virus (CEV), and St. Louis Encephalitis virus (SLE).

Laboratory diagnosis of human arboviral infections at MDCH utilizes an IgM antibody capture ELISA for the detection of arboviral specific IgM antibodies in the serum of patients during the acute phase of infection. Wells of the ELISA plate are coated with anti-IgM antibodies that bind circulating IgM from the patient's serum. Viral antigens from EEE, CEV and SLE are added to different wells and bind to the captured arboviral specific IgM antibodies if present in the patient's serum. The bound antigens are detected with enzyme-linked monoclonal

antibodies specific for the arboviral antigens. The assay is run as a panel on human serum or cerebrospinal fluid. Plasma is acceptable only if drawn in a tube without sodium azide or EDTA. The assay takes three days to perform and is routinely set up Monday through Wednesday, with turn-around time of approximately one week. The laboratory can make accommodations for special circumstances that do not fit into the routine testing schedule. Contact me at (517) 335-8099 or Patty Clark at (517) 335-8102 with questions.

What's New with Flu? Preparing for the Next Pandemic

Duane W. Newton, Ph.D. Director, Virology/Immunology Section

In spite of the uncertainty of timing, there is agreement that the arrival of the next influenza pandemic will present tremendous public health challenges and societal challenges. It is important to develop a preparedness plan in order to successfully face these challenges. MDCH has moved forward in this regard by establishing the Michigan Influenza Pandemic Preparedness Planning Group.

CDC is taking the lead on planning for pandemic influenza and has put together a draft planning guide in conjunction with a number of state and local health departments. This document can be accessed through the website of the National Vaccine Program Office at the CDC (www.cdc.gov/od/nvpo/pandemicflu.htm). Hospitals and local health departments are using this guide to develop plans in their own communities, in cooperation with the state level plan. The Michigan Pandemic Planning Group was borne out of this guide in order for MDCH to develop a state plan, with input from local health departments and the private sector. Participants in the group currently consist of members of the Bureau of Epidemiology and Laboratories at MDCH, county and local health departments throughout the state, the Michigan Society for Infection Control and the Michigan Infectious Disease Society. Using the CDC planning guide as a template, MDCH would like this group to set the broad direction for state planning.

The plan under development is currently organized into four main components: 1) Vaccine and Anti-Viral Delivery; 2) Command, Control and Management/Emergency Preparedness; 3) Surveillance; 4) Communication & Media. Group participants have described their role or their organization's role in addressing issues from each component.

Participants have also formed subcommittees that will function in characterizing elements important for the successful implementation of each component. These elements include but are not limited to: defining the function of each component; identifying individuals or groups with primary and secondary roles in implementing the component and defining their responsibilities; identifying the available and required resources; identifying the legal issues and challenges; and setting the priorities for implementation.

A number of issues need to resolved on a national level, including liability issues for vaccine/drug manufacturers and health care providers. This planning group is establishing the foundation for the statewide response to an influenza pandemic. For more information on the epidemiology and virology of pandemic influenza, see the Winter 1999 edition of EPI Insight from the Bureau of Epidemiology (www.mdch.state.mi.us/pha/epi/ and click on "Bureau Newsletter"). MDCH will continue to provide updates regarding influenza and the Michigan Pandemic Planning Group. Direct questions to me at (517) 335-8099.

Activities Related to Pandemic Influenza Plan Components

Plan Component	Activities Underway/Elements Identified				
Vaccine and Anti- Viral Delivery	Vaccine production has long turn around time, so initial target populations need to be identified				
	State and local HD have experience in mass immunization, vaccine storage and distribution				
	Need to stockpile vaccine and anti- virals for most effective distribution				
Command, Control and Management, Emergency Preparedness	Using Bioterrorism Preparedness as a model				

Surveillance	Sentinel physician surveillance program established, working to improve and expand participation				
	Reporting from hospital labs, schools and daycare centers would enhance the surveillance network				
Communication and Media	Primary contact person required to ensure a unified message				
	Members of media being recruited for assistance				

Submission of Bats for Rabies Testing

Patty Clark, M.P.H. Viral Serology/Viral Isolation Unit

When submitting bats for rabies testing, please do not freeze the bat in order to kill it. Recently, two bats have been received live after being frozen for several hours prior to shipment. Needless to say, this is very hazardous to laboratory personnel. To kill bats prior to testing, MDCH recommends the consultation of a veterinarian for appropriate humane euthanization.

Antimicrobial Resistance Trends, Regions One (Detroit Area) and Two to Twelve (Outstate Michigan) Penicillin Resistant Study-site¹ Isolates of Streptococcus pneumoniae and Vancomycin Resistant Sterile-site² Isolates of Enterococcus spp. Michigan Sentinel Hospital Laboratory Survey, Fourth Quarter, 1995 through Fourth Quarter, 1999

Percent Resistant³

Resistance Microorganism Classification ³	Third/	Quarters Fourth Rg 2-12	First	96 Quarters to Fourth Rg 2-12		Quarters o Fourth g 2-12	First to	Quarters Fourth Rg 2-12	First/ S Rg 1	!999 Qi Second Rg 2-12	Third/ I	Fourth Rg 2-12
S pneumoniae Moderate or Hig	h 20	14	25	18	24	22	21	23	44	48	43	51
S. pneumoniae High Level only	5	4	7	3	11	5	5	7	16	14	18	10
E. faecalis Resistant	1	0	2	1	2	1	3	1	5	1	6	1
E. faecium Resistant	34	7	41	9	49	9	56	40	137	58	135	90
All Enterococcus Resistant	8	1	10	2	13	4	14	7	35	14	31	15

¹ Study sites = blood, CSF, deep surgical wound, pleural fluid(fl.), peritoneal fl., respiratory specimens or synovial fl.

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² Sterile sites = blood, CSF, deep surgical wound, pleural fluid(fl.), peritoneal fl., or synovial fl.

³ NCCLS, Performance Standards for Antimicrobial Susceptibility Testing, M100 - S8.